

BACILLUS ORIGINATED TRANSGLUTAMINASE: PROPERTIES AND USAGE

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Abstract

Enzymes with very important duties, form part of our lives and are useful in various fields. Owing to both the intensity of use and the amount of effective production in standard conditions, the production and use of bacterial-originated enzymes are used continuously in agriculture, health, food and many other industrial areas. Among them, transglutaminases (EC 2.3.2.13) are both intracellular and extracellular enzymes included in the transferase group that catalyse cross-links between proteins. Microbial transglutaminase enzymes are frequently used in the food and pharmaceutical industries to change the functional properties of proteins since it increases viscosity, elasticity and water holding capacity. Particularly the positive effects in meat, dairy, and bakery products such as gelling, increasing mechanical strength, and reducing structural deformations, specifically the texture. It also contributes to reducing the use of additives in diets with low protein and fat content. Furthermore, it reduces the time for cooking processes as well as sensory properties enhancement. Transglutaminase enzymes are also being used in other fields such as tissue culture, biochemical and biomedical research, textile, and leather industries. In this review, a broad perspective is presented on the literature dealing with bacterial transglutaminase studies, especially those belonging to the genus *Bacillus*. In *Bacillus* spp., transglutaminase gene was mostly reported in *B. subtilis*, *B. amyloliquefaciens*, *B. cereus*, *B. nakamura*, *B. circulans* species and also recently in the whole genome of local *Bacillus thuringiensis* (*Bt*) SY49.1 strain (JAHKEZ010000474.1) using the RAST database. It was determined that the *Bt* transglutaminase gene was 93% identical to that of *B. cereus* species. The presence of transglutaminase gene in agriculturally indispensable *Bt* strain can confer them a superficial characteristic due to their water holding capacity in terms of increasing the yield and quality of plant products.

Keywords: *Bacillus* spp., Bacterial originated enzymes, Transglutaminase

1. TRANSGLUTAMINASE

Transglutaminase enzyme (TGase); (EC 2.3.2.13) is one of the enzyme types belonging to the transferase group found in animals, plants and microorganisms. TGase provides protein modification by forming cross-links of glutamine and lysine amino acids in the protein chain (Duarte et al., 2020). According to the organism from which it is obtained, the TGase enzyme is called structural TGase if it is of animal or vegetable origin, and microbial TGase if it is of microbial origin (Serdaroğlu and Turp, 2003). The isoelectric point of the microbial transglutaminase enzyme (MTGase) is around pH 8.9. Its molecular weight was measured by both

SDS-PAGE and chromatographic methods and was found to be approximately 38 kDa. The primary structure of the protein sequence of MTGase was determined by the Edman method and mass spectrophotometry methods and it was determined to consist of approximately 331 amino acids (Yokoyama et al., 2004).

TGase enzyme catalyzes the transfer of groups carrying C, N, P molecules and the formation of cross-linking reactions of lysine and glutamine amino acids. Its mode of activity involves the transamidation between the primary amine group of lysine residue and the carboxamide moiety of a glutamine (Roshental et al., 2013). It catalyzes the interconnections of myofibrils and creates a protein-protein network (Ribeiro et al., 2021). The reactions catalyzed by the TGase enzyme are shown in Figure 1 and the working principle is shown in Figure 2.

1) Acyl transfer reaction



2) Cross-linking reaction between Gln and Lys residues of proteins or peptides



3) Deamidation



Figure 1. Reactions catalyzed by the enzyme transglutaminase

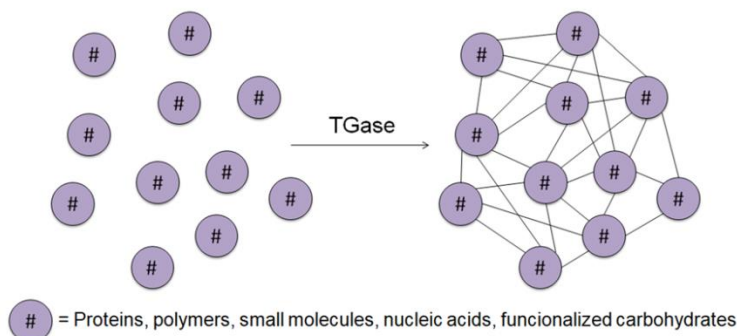
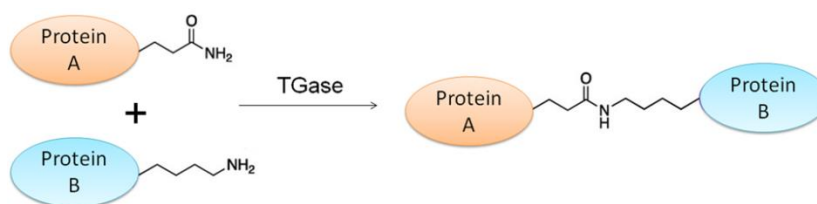


Figure 2 Working principle of transglutaminase enzyme

- 1) It catalyzes the acyl transfer reaction between the γ -carboxamide in the structure of peptide or protein-bound glutamine and the primary amine.
- 2) It catalyzes the formation of ϵ -(γ -Glu) Lysine cross-linking between glutamine and lysine amino acids.
- 3) It catalyzes the use of water in case there is no suitable primary amine in the environment or the ϵ -amine group of lysine is bonded with certain agents (Eren Karahan, 2015).

2. USAGE AREAS OF TGase

Although transglutaminase has various uses such as pharmaceuticals and textiles, the most common area of use is food processing. TGase is commonly used for improving the nutritional value and rheological properties of food. It provides positive changes in the texture of the food because it catalyzes the cross-links between proteins.

As a result of the reactions catalyzed by TGase, it has special functional properties including;

- Gelation in low viscosity foods (proteins that do not gel with heat may gel),
- Increasing the water holding capacity,
- Increasing the foaming capacity,
- Emulsifying
- Increasing mechanical durability,
- Contributing to the mechanical structure of products containing low fat and protein,
- Balancing the amino acid deficiency in the existing structure,
- Reducing the deformation in the texture of the food,
- Adding functional properties to food,
- Improving sensory features,
- Features such as reducing the use of food additives occur.

Because it provides these properties to food, it is used especially in meat and meat products, milk and dairy products, aquatic products and bakery products (Yüksel and Erdem, 2006).

2.1. Usage of TGase in Dairy Products

It is known that the casein protein in milk is a good substrate for TGase. It has been observed that milk casein, which does not form a gel with heat, forms a gel with TGase activity (Akbari et al., 2021). TGase enzyme is used in dairy products to increase yield in cheese with its water-holding capacity, to reduce syneresis in yoghurt with its structural strength, and to improve crystal structure in ice cream with its emulsifying feature. It is used especially in industrial problems experienced in products with reduced oil content, as it increases gel stability and final product stability. At the same time, various studies have been carried out on other dairy products such as kefir and ayran. In a study conducted on kefir, it was determined that TGase contributes to the sensory properties of kefir. Aromatic substances that improve taste and smell have been found. At the same time, it has been determined that casein fractions reduce immune reactivity (Eren Karahan, 2015).

2.2. Usage of TGase in Meat Products

It is known that the binding between proteins in processed meat products is very important for the product. Studies have shown that myosin gelation plays a very important role in the binding of proteins and the quality of the product's texture. It has been observed that the TGase enzyme catalyzes the covalent cross-linking between myosin and the filler additives used (Serdaroğlu and Turp, 2003).

In a study on the effect of papain and TGase enzyme on the textural properties of beef and chicken burgers, it was determined that when pH enzymes are used together, it gives softness to both products (Ribeiro et al., 2021). In another study the effects of TGase on the quality characteristics of different types of pastrami, the maximum shear force, water activity and free amino acid composition of the samples were investigated. TGase increased the cutting force while decreasing the aw value. At the same time, an increase in the level of free amino acids was observed (Hazar et al., 2021).

3. BACTERIAL PRODUCTION OF TGASE

Enzymes are non-toxic biocatalysts and their use increases production efficiency while reducing processing time and energy use (Duarte et al., 2020). Developing enzyme technologies have made it possible to produce enzymes that are used in industry in a purer, low cost and more abundant amount. Recombinant technology using the microorganisms is one of the top priority methods to meet the growing need for enzymes at low costs (Serin, 2014). The reason for the widespread use of microbial enzymes is that their catalytic activities are higher than those of plant and animal origin, they do not form unwanted by-products and they are more stable (Eren Kiran et al., 2006). These applications have enabled us to have the chance to select not only according to the ability of microorganisms to produce enzymes but also according to their non-toxicity and pathogenicity (Duarte et al., 2020).

TGase enzyme is a natural enzyme with substrate specificity, which is known to be produced by many microorganisms as well as being found in animal and plant tissues. Depending on the macromolecular structure of the protein, whether it is in the flexible region of the polypeptide chain or the reversed region of the TGase enzyme obtained from different sources, the rate of binding with the substrate varies (Gauche et al., 2008).

The first microbial TGase enzyme was detected in *Streptomyces* sp. by Ando et al. in 1989. They reported that, unlike TGase of animal origin, it is independent of Ca^{2+} and has a smaller size (Ando et al., 2014). The optimum pH range of microbial TGases is 5-8, the temperature range at which they show enzymatic activity is 10-70 °C, and the optimum temperature is 50 °C (Motoki and Seguro, 1998).

Lin et al., in a study on bacteria, cloned TGase gene from *Streptomyces platensis* M5218 and transferred to *Streptomyces lividans*. They determined that the open reading frame of MTGase gene was 1254 nucleotides long and encoded a protein consisting of 418 amino acids with 46 kDa. The amino acid sequence of the protein was identical to those of *Streptovorticillium spp* at a level of 69.3-77.7%. Analysis with immunoblotting and SDS-PAGE revealed the presence of TGase in culture supernatant of transfected strain. They concluded that recombinant MTGase can be expressed in high amount and processed during secretion in transformed *S. lividans* JT46 (Lin et al., 2006).

Microbial pro-TGase from *Streptomyces mobaraensis* was cloned by Marks et al. and expressed for the first time as a highly soluble protein in *Escherichia coli*. According to SDS-PAGE, more than 90% of TGase was produced in soluble form (Fatima et al., 2021). Liu et al. cloned the TGase gene encoded by *Streptomyces fradiae* by inserting another copy of the TGase gene into the original host genome, which was driven by the strong constitutive promoter "ermE up" and was shown to be expressed at mRNA and protein levels. It was indicated that the activity (3.2 U/ml) of TGase in recombinant strain rised up to 1.3-fold compared with source strain (2.4 U/ml). However, specific

activity of the enzyme (3.8 U/mg) in recombinant strain was twice that of the source organism (1.9 U/mg) (Liu et al., 2006).

In another study, the *Streptomyces hygroscopicus* pro-TGase gene was inserted into the integrative vectors pINA1296 (monocopy) and pINA1297 (multicopy) and transfected to *Y. lipolytica* Po1g or Po1h strain, respectively. The expression was accomplished under a recombinant hp4d promoter. XPR2 pro-sequence was used as signal peptide. Recombinant Po1h strain produced the highest amount of extracellular pro-TGase (5.3 U/mL). This amount was 8.8 times higher than that obtained in recombinant Po1g strain (Liu et al., 2015).

Liu et al. cloned TGase gene fragment together with its promoter, signal peptide and terminator regions from the source organism *Streptomyces hygroscopicus* WSH03-13 into pIJ86 vector. Transfected *Streptomyces lividans* TK24 produced 1.8 U/mL of TGase. Electrophoresis result proved the presence of 38 kDa TGase in the culture supernatant. These results demonstrated successful expression of pro-TGase and correct cloning of active TGase in *S. lividans* TK24 using the TGase promoter (Liu et al., 2016). Washizu et al. designed oligonucleotides using the TGase sequence from *Streptoverticillium mobaraense* and amplified a gene fragment. The gene encoded a TGase precursor of 406 amino acids containing the prepro region of 75 amino acid residues and the mature region of 331 amino acid residues. The recombinant enzyme TGase in *Streptomyces lividans* was expressed under a tyrosinase promoter (Washizu et al., 2014).

In a study, Ando et al., purified microbial TGase from the culture filtrate of S-8112 strain, thought to be of the genus *Streptoverticillium*. It was found that the molecular weight of the enzyme purified in SDS-PAGE gel electrophoresis is about 40 kDa with isoelectric point of 8.9. Optimal pH of the reaction was around 6-7. It has been determined that the activity of the present enzyme does not require calcium ions. They reported that this feature is different from the animal-derived TGase enzyme, which is active in the presence of calcium (Ando et al., 1989). Salis et al. investigated the production of recombinant TGase from *Streptomyces mobaraensis* in *E. coli* using different expression systems under the control of both the lac promoter and the thermo-inducible phage lambda promoter. Considering the experimental data on the expression levels and specific activities of purified MTGase proteins, the gene encoded LacZ1-8Met-MTGase using plasmid PL463 with an enzymatic activity comparable to the commercial enzyme was determined as the most effective strain for the production of MTGase for industrial applications (Salis et al., 2015). Yu et al., purified the MTGase from *Streptomyces netropsis* BCRC 12429 strain. The molecular weight of the purified TGase was determined to be approximately 38 kDa by SDS-PAGE gel electrophoresis analysis. The TGase gene of *S. netropsis* was cloned and a 1,242 bp open reading frame encoding a 413 amino acid protein was identified. TGase was synthesized as a pre-regional precursor protein of 82 amino acid residues. The identified amino acid sequence of *S. netropsis* TGase was expressed in *E. coli* in a highly soluble form. The purified recombinant protein was found to have an equivalent activity to the control TGase (Yu et al., 2008).

4. BACILLUS ORIGINATED TGASE

Obtaining the transglutaminase enzyme from *Bacillus* species was realized in 1996. It is a 28 kDa protein with a single-domain produced in active form. It also catalyzes the formation of reactions that form protease-resistant and stable ϵ -(γ -glutamyl)-lysine cross-links. It has been reported to have a more stable structure when compared to *Streptoverticillium mobaraense* TGase (Zilhao et al., 2005).

Microbial TGase, gene constructs encoding TGase, methods for the production of TGase, and the presence of biochemically active TGase in *B. mycoides*, *B. Firmus*, *B. aneurinolyticus* *B. megaterium*, *B. badius*, *B. Amyloliquefaciens* have been reported. Strategies can be developed for more efficient production in this direction (Bech et al., 2006). Kobayashi et al. screened various *Bacillus* species and reported TGase activity in spore-forming cells of *B. subtilis*, *B. cereus*, *B. alvei* and *B. aneurinolyticus*. TGase was found to be localized on spores. Importantly, TGase activity is associated with *Bacillus* sporulation process (Kobayashi et al., 1996). They also reported that the gene encoding TGase was cloned in *B. subtilis* and expressed in *E. coli*. They reported that TGase activity was detected in *E. coli* cells transformed with a plasmid for expression of the TGase gene (Kobayashi et al., 1998).

Gohar et al., compared the extracellular enzymes of *Bacillus* species, determined the presence of TGase in *Bacillus cereus* and *Bacillus thuringiensis* strains (Gohar et al., 2005). Duarte et al., aimed to transfer TGase gene from *Bacillus amyloliquefaciens* and obtained the active form of the protein in *E. coli*. They constructed a bicistronic vector including *B. amyloliquefaciens* TGase gene fused with the inhibitor *Streptomyces caniferus* prodomain for reducing the possible toxicity of TGase in host cells. They also cloned the 3C protease gene in the same plasmid to activate the enzyme. After purification, they obtained a partially purified recombinant TGase with 37 mU/mg protein activity cross-linked with bovine serum albumin (BSA). In conclusion, *B. amyloliquefaciens* TGase was expressed in its mature and active form in *E. coli* (Duarte et al., 2020).

Wang et al., cloned the TGase gene from *Streptomyces sp.* and expressed in *Bacillus subtilis* SCK6. Codon and signal peptide (SP Ync M) optimisation was done on MTC. The activity of the extracellular enzyme was reported to be 20 times higher than that of the previously optimized enzyme. MTGase was 38 kDa with the specific activity of 63.75 U/mg. Gelatin crosslinked with MTGase increased the strength of the gelatin 1.67-fold and increased the thermal denaturation temperature from 61.8 to 75.8°C. It was also evident that strength and thermal stability of gelatin was considerably increased. These are some of the characteristics of of MTGase with great commercial potential as applications in salty protein foods (Wang et al., 2020). In light of this information, the TGase gene was screened in some *Bacillus species* using the RAST database. Taking the the TGase protein of *Bacillus cereus* F837/76 as reference, whole genome of local *Bacillus thuringiensis* SY49.1 strain was screened and found that it bears the gene with 93% similarity.

5. USAGE OF TGase IN AGRICULTURE

Not many studies have been reported on the use of TGase enzyme in agriculture, and its application to plants and roots and examination of quality parameters are considered among future studies in terms of increasing water holding capacity. It is thought that if the TGase gene in agriculturally indispensable *Bt* strain is modified and its expression is increased, then it can give them a new feature due to their water-holding capacity in terms of increasing the yield and quality of plant products.

6. CONCLUSION

In this review, a broad perspective is presented on the literature dealing with bacterial TGase studies, especially those belonging to the *Bacillus genus*. Although the TGase gene has been determined in different bacterial species in various studies, few research is present on *Bacillus*

species TGase production. We determined the presence of TGase gene in local *Bacillus thuringiensis* SY49.1 strain and it was recommended that molecular and chemical studies to improve this gene's products may confer a superior feature to these agriculturally indispensable bacterial strains to be used in increasing the crop yield.

7. REFERENCES

- Akbari, M., Razavi, S.H., Kieliszek, M. (2021). Recent advances in microbial transglutaminase biosynthesis and its application in the food industry. *Trends in Food Science & Technology*, 110, 458-469.
- Ando, H., Adachi, M., Umeda, K., Matsuura, A., Nonaka, M., Uchio, R., Tanaka, H., Motoki, M. (2014). Purification and characteristics of a novel transglutaminase derived from microorganisms. *Agricultural Biological Chemistry* 53 (10), 2613–2617.
- Duarte, L.S., Barse, L.Q., Dalberto, P.F., Silva, W.T.S., Rodrigues, R.C., Machado, P., Basso, L.A., Bizarro, C.V., Ayub, M.A.Z. (2020). Cloning and expression of the *Bacillus amyloliquefaciens* transglutaminase gene in *E. coli* using a bicistronic vector construction. *Enzyme and Microbial Technology*, 134(2020) 109468.
- Eren Karahan, L. (2015). Microbial transglutaminase enzyme and its use in dairy products. *Batman University Journal of Life Sciences*, 5(2), 200-215.
- Eren Kiran, Ö., Çömləkçiođlu, U., Dostbil, N. (2006). Some microbial enzymes and their use in industry. *KSU. Journal of Science and Engineering*, 9(1), 12-19.
- Fatima, S.W., Khare, S.K. (2021). Effect of key regulators in augmenting transcriptional expression of Transglutaminase in *Streptomyces mobaraensis*. *Bioresource Technology*. 340, 125627.
- Gauche, C., Vieira, J., T.C., Ogliari, P., J., Bordignon-Luiz, M., T., (2008). Crosslinking of milk whey proteins by transglutaminase, *Process Biochemistry* 43, 788–794.
- Gohar, M., Gilois, N., Graveline, R., Garreau, C., Sanchis, V., Lereclus, D. (2012). A comparative study of *Bacillus cereus*, *Bacillus thuringiensis* and *Bacillus anthracis* extracellular proteomes. *Proteomics* 5, 3696–3711.
- Hazar, F.Y., Kaban, G., Kaya, M. (2021). The effects of transglutaminase on the qualitative properties of different pastirma types. *LWT - Food Science and Technology* 145, 111289.
- Kobayashi, K., Kumazawa, Y., Miwa, K., Yamanaka S. (1996). ϵ -(γ -Glutamyl)lysine cross-links of spore coat proteins and transglutaminase activity in *Bacillus subtilis*. *FEMS Microbiology Letters*, 144, 157-160.
- Kobayashi, K., Hashigucci, K., Yokozeki, K., Yamanaka, S. (1998). Molecular Cloning of the Transglutaminase Gene from *Bacillus subtilis* and Its Expression in *Escherichia coli*. *Biosci. Biotechnol. Biochem.*, 62 (6), 1109–1114.
- Kumari, A., Kritika Singh, K., Kayastha, A.M. (2012). α -Amylase: general properties, mechanism and biotechnological applications – A Review. *Current Opinion in Biotechnology*, 1: 98-107.
- Lin, Y., Chao, M., Liu, C., Tseng, M., Chu, W. (2006). Cloning of the gene coding for transglutaminase from *Streptomyces platensis* and its expression in *Streptomyces lividans*. *Process Biochemistry* 41, 519–524.
- Liu, X., Fuhong X.Y., Qian X.S. (2006). Cloning of transglutaminase gene from *Streptomyces fradiae* and its enhanced expression in the original strain. *Biotechnol Lett*, 28, 1319–1325.
- Liu, S., Wan, D., Wang, M., Madzak, C., Du, G., Chen, J. (2015). Overproduction of pro-transglutaminase from *Streptomyces hygroscopicus* in *Yarrowia lipolytica* and its biochemical characterization. *BMC Biotechnology* 15:75.
- Liu, S., Wang, Du, G., Chen, J. (2016). Improving the active expression of transglutaminase in *Streptomyces lividans* by promoter engineering and codon optimization. *BMC Biotechnology* 16:75.
- Motoki, M. and Seguro, K. (1998). Transglutaminase ve its use for food processing. *Trends in Food Science ve Technology*. 9, 204-210.
- Ribeiro, W.O., Ozaki, M.M., Santos, M., Rodríguez, A.P., Pflanzler, S.B., Pollonio, M.A.R. (2021). Interaction between papain and transglutaminase enzymes on the textural softening of burgers. *Meat Science* 174, 108421
- Rosenthal, M.D., Glew, R.H. (2011). *Human Metabolism in Health and Disease. Medical Biochemistry*. 13-15.
- Salis, B., Spinetti, G., Scaramuzza, S., Bossi, M., Jotti, G.S., Tonon, G., Crobu, D., Schrepfer, R. (2015). High-level expression of a recombinant active microbial transglutaminase in *Escherichia coli*. *BMC Biotechnology* 15:84.
- Serdarođlu, M., Turp, G.Y. (2003). Use of transglutaminase in food processing. *Food* 28(2), 209-215.
- Serin, B., 2014. Cloning and characterization of α -amylase from *Bacillus subtilis* atcc 6051. Dicle University, Diyarbakir.

- Wang, S., Yang, Z., Li, Z., Tian, Y. (2020). Heterologous Expression of Recombinant Transglutaminase in *Bacillus subtilis* SCK6 with Optimized Signal Peptide and Codon, and Its Impact on Gelati Properties. *Journal Microbiology and Biotechnology* 30(7): 1082–1091.
- Washizu, K., Ando, K., Koikeda, S., Hirose, S., Matsuura, A., Takagi, H., Motoki, M., Takeuchi K. (2014). Molecular Cloning of the Gene for Microbial Transglutaminase from *Streptovercillium* and Its Expression in *Streptomyces lividans*. *Bioscience, Biotechnology, and Biochemistry*, 58 (1),82-87, 1994.
- Yokoyama K., Nio N., Kikuchi Y. (2004). Properties and application of microbial transglutaminase. *Appl Microbiol Biotechnol* 64: 447–454.
- Yu, Y.J., Wu, S.C., Chan, H.H., Chen, Y.C., Chen, Z.Y., Yang M.T. (2008). Overproduction of soluble recombinant transglutaminase from *Streptomyces netropsis* in *Escherichia coli*. *Applied Genetics And Molecular Biotechnology*, 81:523–532.
- Yüksel, Z., Erdem, Y.K. (2006). Applications of Transglutaminase in the Food Industry: Uses of the 2nd Enzyme in Food Processes. *Food*, 33 (3) : 143-149.
- Zilhão, R., Istatico, R., Martins, L.O., Steil, L., Völker, U., Ricca, E., Moran Jr., C.P., Henriques, A.O. (2005). Assembly and function of a spore coat-associated transglutaminase of *Bacillus subtilis*. *J. Bacteriol.* 187 (22) 7753–7764.